Determination of contents of 10-Hydroxycamptothecin in *Camptotheca acuminata* by high-performance liquid chromatogram

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Abstract: The determination method of 10-hydroxycamptothecin in *Camptotheca acuminata* fruits by high-performance liquid chromatogram (HPLC) was studied. The HPLC analysis was performed on a HIQ sil C_{18}(4.6×250 mm) column with mobile phase of acetonitrile–water (3:7, V:V), flow rate 1 mL··min^{-1} and UV detective wavelength 266 nm. Extracting 10-hydroxycamptothecin by ultrasonic method from fruits of *C. acuminata* to prepare samples for analysis was systematically discussed. The optimal extraction condition was carried out by 60% alcohol solution at 60℃ for 50 minutes.

Key words: 10-hydroxycamptothecin; *Camptotheca acuminata*; HPLC; ultrasonic extraction method
CLC number: O652.63 **Document code:** B **Article ID:** 1007-662X(2002)02-0144-03

Introduction

Camptotheca acuminata Decaisne (Nyssaceae) is a native tree species in the south of China. Wall *et al.* first isolated the Camptothecin (CPT), an indole alkaloid, from the wood in 1966 (Wall *et al.* 1996; Wall and Wani 1991; Helmut *et al.* 1997). 10-Hydroxycamptothecin (HCPT) is a natural derivative of CPT, mainly existing in fruits of *C. acuminata*. It has the same strong anticancer activity as CPT in inhibiting the activity of topoisomerase (Slichenmyer and van Hoff 1990, Liu and Adams, 1995). HCPT has been used to cure liver carcinoma, urinary bladder carcinoma, and colorectal cancer, etc. in China (Sun *et al.* 1999). The determination of HCPT by HPLC (High performance liquid chromatogram) was studied in recent years. However the extraction of HCPT from *C. acuminata* fruits for analysis has not been referred so far (Teng and Jiang 1997). Ultrasonic extraction is a rapid, simple and accurate method for preparation of analyzing samples. In this paper, the optimal extraction method for the preparation of analyzing sample by HPLC was systematically studied.

Materials

Apparatus: JASCO HPLC system with UV-1575 detector; Biofuge 22R high speed centrifugal machine; B104 electronic balance; B-321 ultrasonic oscillator; Multitemp III cycle water-bath.

Reagent: Acetonitrile is chromatographically pure; alcohol is analytically pure.

Standard samples: HCPT was bought from Donglan Pharmaceutical Plant, Guangxi Province, and its purity is 95%.

Plant material: C. acuminata fruits, sampling from Jintang, Sichuan Province, was dried at 80℃ for 24 h, crushed and screened by 60 mesh, and after then stored in the desiccator.

HPLC analysis

Condition of HPLC analysis

HIQ sil C_{18} column (4.6×250 mm, Japan); mobile phase acetonitrile–water (3:7, V:V); flow rate 1 mL·min^{-1}; sample loop 10 μL; room temperature.

Wavelength

We selected 266nm as the absorbent wavelength by scanning the alcohol solution of the HCPT standard sample from 200 nm to 800 nm by spectrophotometer.

Standard solution and standard curve

1.2-mg HCPT was dissolved in 100-mL alcohol at first, after then we injected 1-mL, 2-mL, 3-mL, 4-mL, and 5-mL dissolved solution separately into the five 10-mL measuring flasks, and add alcohol to the standard scale. All samples were analyzed in triplicate by HPLC. The standard curve was set up between concentration and peak areas

$$Y = 32391X + 5937.2$$

Here X is the HCPT concentration, Y is the peak area, R=0.9969: the linear range is 1.2~6.0 μg·mL^{-1}.

Preparation of analyzing samples

Optimal conditions for the extraction of HCPT from *C. acuminata* were studied by using the uniform design of three factors and 11 levels. The three factors were concentration of alcohol, extracted temperature, and extracted time. One gram fruit powder was dissolved in 15-mL alco-

Foundation Item: This paper was supported by National Natural Science Foundation of China (No. 30070086).
Biography: MA Mei-fang (1976-), female, Graduate Student of Northeast Forest University
Received date: 2002-01-12
Responsible editor: Song Funan

hol solution, and extraction was carried out by ultrasonic method at the corresponding temperature for certain time. When he extracting solution was cooled to room temperature, we took out 1-mL supernatant to centrifuge at 12 500 rpm for 10 min, after than the determination of the HCPT content of *C. acuminata* was conducted by HPLC. The experimental conditions and results were shown in Table 1.

Table 1. $U_{11}(11^3)$Experimental conditions and results by uniform design (Deviation: 0.1847)

Sample No.	Extracted temperature/℃	Extracted time /min	Concentration of alcohol /%	Yield of 10-hcpt /‰	Fitted value	Residual value
1	10	55	40	0.0574	0.05635	0.00105
2	15	40	80	0.0509	0.04732	0.00358
3	20	20	50	0.0475	0.05602	-0.00850
4	25	25	20	0.0322	0.02868	0.00352
5	30	5	30	0.0477	0.04701	0.00069
6	35	15	90	0.0204	0.02772	-0.00472
7	40	45	100	0.0230	0.02443	-0.00140
8	45	50	10	0.0274	0.02896	-0.00160
9	50	10	60	0.0830	0.07896	0.00404
10	55	30	70	0.1033	0.10442	-0.00110
11	60	35	0	0.0281	0.02837	-0.00030

Equation of the optimal regression is established by uniform design software as follows:

$$Y = -0.0980952 + 0.0023194X_1 + 0.0041432X_3 - 0.0000126X_1X_3 - 0.0000104X_2^2 + 0.0000352X_2X_3 - 0.0000471X_3^2$$

Here X_1 is the extracted temperature (℃), X_2 is the extracted time (min), and X_3 is the concentration of alcohol (%). The verified value F=24.3358>$F_{0.05}$=8.9400 indicates the regression equation is significant. The multiple correlation coefficient of the regression is 0.9899, residual standard deviation (*s*) 0.0064, and residual sum of squares (*SE*) is 0.0001. The results are showed in Table 1.

The influence of each factor on yield of HCPT is shown as Fig.1-3. The determined value of HCPT increased with the increase of the extracted temperature and extracted time. However the influence of alcohol concentration on determination value of HCPT showed a single peak curve, from which it is known that the optimal concentration of alcohol is 60%. Consequently, extracting with 60% alcohol solution, in 50 minutes extracting, and at a temperature of 60 ℃ can be the optimal conditions for extraction of HCPT.

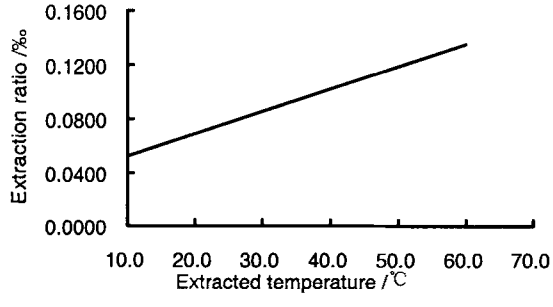

Fig. 1 Influence on the yield of HCPT by extracted temperature

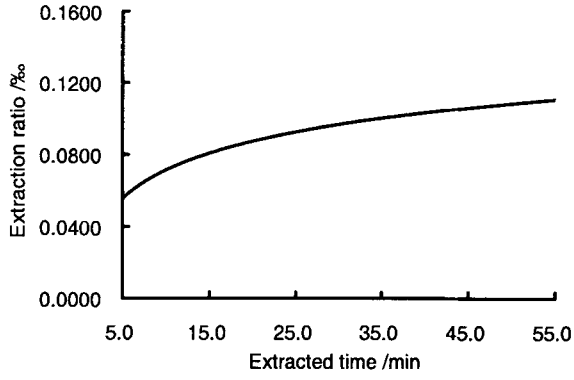

Fig. 2 Influence on the yield of HCPT by extracted time

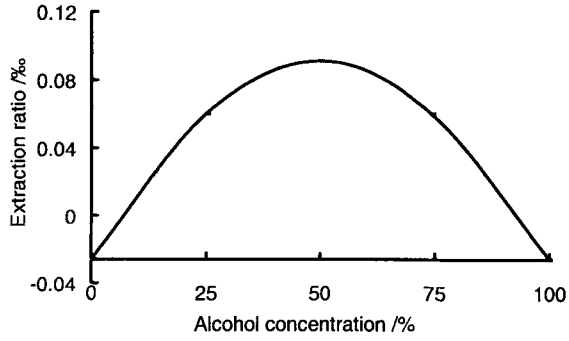

Fig. 3 Influence on the yield of HCPT by alcohol concentration

Precision degree

We put separately four 1-g fruit powder into 25-mL alcohol solution. and extracted HCPT under the optimal conditions mentioned above. The extracting solution was analyzed by HPLC. All samples were analyzed in tricubic. HCPT concentration is 0.1‰, RSD is 5.04%.

Recovery coefficient

We put separately six 0.5-g fruit powder into 25-mL alcohol solution, and added 1-mg standard sample of HCPT in it. The samples were extracted and detected under the above conditions. The average recovery rate is 93.56%.

Conclusion

The HPLC conditions for analyzing HCPT are 266-nm detective wavelength mobile phase acetonitrile-water (3:7, V:V), flow rate of 1 $mL \cdot min^{-1}$, and room temperature. The optimal conditions for the preparation of analysis samples were that the concentration of alcohol solvent is 60%, the extracted time 50 min, and the extracted temperature 60℃. The HCPT concentration in *C. acuminata* fruits is 0.1‰. The RSD is 5.04%, and the average recovery rate is 93.56%. This method is simple, rapid and feasible for the analysis of HCPT.

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